

Germination and Early Seedling Growth of Chaffy-Seeded Grasses at Negative Water Potentials

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ABSTRACT

Drought is the most frequent cause of stand establishment failure of native grasses. An experiment was conducted to determine the interactions of seed processing and simulated dry conditions on seed germination and seedling growth of five chaffy-seeded, warm-season grasses. Seed of big bluestem (*Andropogon gerardii* Vitman), sand bluestem (*Andropogon hallii* Hack.), little bluestem [*Schizachyrium scoparium* (Michx.) Nash], yellow bluestem [*Bothriochloa ischaemum* (L.) Keng. var. *ischaemum* (Hack.) Celarier and Harlan], and indiangrass [*Sorghastrum nutans* (L.) Nash] were processed into intact spikelets-chaffy pure-seeds, debearded spikelets-chaffy pure-seeds lightly trimmed of hair and awns, and caryopses. Fifty-seed samples of each species and seed form were germinated at water potentials of -1.6, -0.8, -0.4, -0.2, and 0 MPa for a 21-d period. There were species \times water potential and species \times seed form interactions for 7-d germination, 21-d germination, and potential maximum germination, and for seedling root and shoot lengths. In general, seed germination and seedling growth were reduced as the water deficit potentials increased. Germination was slower and much lower from intact and debearded spikelets compared with caryopses. The slower germination of intact and debearded spikelets also resulted in shorter root and shoot lengths when compared with caryopses. Given the amount of genetic variation within most cross-pollinated species, this procedure could be used as an effective selection tool for breeding cultivars with improved germination and seedling growth at low water potentials.

SEVERAL FACTORS influence seed germination, early seedling growth, and associated stand establishment of warm-season pasture grasses. Such factors include inadequate seed-bed preparation, improper seeding depth, late seeding date, inferior seed quality, competition from weeds, crusting of soil surface, and drought. Of these, drought is undoubtedly the most frequent cause of plant establishment failure (Blake, 1935).

The inherent nature of the seeding unit can also cause difficulty in the proper placement of seed. The term "chaffy-seed" denotes seed units consisting of a caryopsis and its subtending appendages (lemma, palea, glumes, hairs, and awns). Chaffy grass seed are inherently more difficult to store and to plant than non-chaffy seed. Removal of the chaffy appendages eases planting difficulties but can limit the longevity of seeds in storage even under the best of conditions (Ahring, 1962). Seed of

chaffy-seeded grasses can be debearded, i.e., removal of the hairs and awn (Ahring et al., 1964) or processed to remove all appendages leaving only caryopses (Beisel, 1985). When conditions are favorable, planting caryopses offer lower seeding rates and more uniform seedling emergence, but during unfavorable conditions caryopses are more susceptible to attack from microorganisms (Rollins and Ahring, 1987). Chaffy seed tend to have greater seed dormancy than more heavily processed seed, allowing for earlier planting in less favorable conditions (Springer, 1991).

Several experiments have simulated the effects of moisture stress on seed germination and growth of plants (Helmerick and Pfeifer, 1954; McGinnies, 1960; Parmar and Moore, 1968; Knipe, 1973; Sharma, 1973, 1976). These studies used a number of chemical compounds to simulate water deficits, such as, carbowax 6000, D-mannitol, glucose, NaCl, sucrose, polyethylene glycol (PEG), and polyvinylpyrrolidone. The most commonly used compounds were mannitol and polyethylene glycol because they are considered chemically inert and nontoxic to plant growth (Parmar and Moore, 1968; Bell, 1974).

The interactions of seed processing with plant species, germination under moisture stress, and early seedling growth are not well defined. Seed processing affects the rate of germination of chaffy-seeded grasses (Ahring et al., 1964; Springer, 1991). Similarly, moisture stress decreases seed germination and seedling growth (Helmerick and Pfeifer, 1954; Wiggins and Gardner, 1959; Parmar and Moore, 1968). Thus, the objective of this experiment was to determine the interactions of seed processing and moisture stress on the germination and seedling growth of five chaffy-seeded, warm-season grasses.

MATERIALS AND METHODS

The chaffy-grass seed used for study were representative of plant materials available to producers. Chaffy seed of 'Rountree' big bluestem, 'Osage' indiangrass, and 'Aldous' little bluestem were purchased from commercial sources. Chaffy seed of 'WW-Spar' yellow bluestem, and 'Chet' sand bluestem were produced at the USDA, ARS, Southern Plains Range Research Station, Woodward, OK.

The chaffy seed of each grass species was blown with the aid of a Seedburo seed blower (Seedburo, Chicago, IL) at air valve openings of 30, 27, 30, and 22 mm for indiangrass, little bluestem, big and sand bluestem, and yellow bluestem, respectively. Each seed lot was blown in 3-g increments to remove all light and empty chaffy seed units. The chaffy pure-seed (consisting of a caryopsis and its subtending appendages) content of the heavy seed fraction of this blowing technique was 98% ($\pm 1\%$) for each species. Thirty grams pure seed of each species was obtained in this manner and assigned to one of the following treatments: (i) intact spikelets-chaffy pure-seeds, (ii) debearded spikelets-rough pure-seeds lightly trimmed of

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Table 1. Fifty seed weight (mean \pm SE) for caryopses, debearded spikelets, and intact spikelets for WW-Spar yellow bluestem, Aldous little bluestem, Osage indiagrass, Rountree big bluestem, and Chet sand bluestem.

Cultivar	Caryopses	Debearded spikelets	Intact spikelets
		mg	
Yellow bluestem	22.7 \pm 0.4	32.3 \pm 0.4	36.1 \pm 0.5
Little bluestem	65.0 \pm 2.0	86.1 \pm 2.0	102.4 \pm 3.5
Indiagrass	97.8 \pm 1.1	139.2 \pm 1.2	144.9 \pm 2.8
Big bluestem	99.6 \pm 1.7	146.5 \pm 2.6	162.4 \pm 2.6
Sand bluestem	116.3 \pm 3.2	183.5 \pm 3.9	209.6 \pm 6.0

hair and awns with the aid of a rub-board, and (iii) caryopses—processed with the aid of a Woodward laboratory air-seed shucker (Ag-Renewal, Inc., Weatherford, OK). Shucked seed was hand picked under $5\times$ magnification to remove all caryopses with apparent damage. Only apparently undamaged caryopses were used in germination tests. Because undamaged caryopses were selected, it is unlikely that caryopses extracted by other methods, e.g., by hand, etc., would be of better quality.

Forty, 50-seed samples of each species and seed form were counted from the 30-g sample of processed seed (outlined above), weighed, and retained for germination tests. Fifty seed weight averaged from 22.6 to 116.3 mg for caryopses, from 32.3 to 183.5 mg for debearded spikelets, and from 36.1 to 209.6 mg for intact spikelets (Table 1).

For each germination test, 20 of the 50-seed samples of each species and seed form were randomly assigned to one of five water potential treatments. Water potential treatments of -1.6 , -0.8 , -0.4 , -0.2 , and 0 MPa were prepared by mixing 58.3, 29.2, 14.6, 7.3, and 0 g D-mannitol in 0.5 kg of deionized water, respectively. Deionized water was used as a control, water potential = 0 MPa. D-Mannitol was chosen because it acts as an inert osmotic medium (Parmar and Moore, 1968; Bell, 1974). Fifty seeds of each species and seed form were placed in sterile, clear plastic boxes ($7.0 \times 7.0 \times 2.5$ cm) on two layers of absorbent paper towel substrates moistened with 7 mL of each water potential solution. Germination was conducted in a seed germinator (Stultz Scientific Engineering Corp., Springfield, IL) set for 8 h d^{-1} of fluorescent light at 30°C and 16 h d^{-1} of darkness at 20°C . Cumulative normal germination counts were made at 7, 14, and 21 d (Colbry et al., 1961). At the end of 21 d, the number of firm (nongerminated, apparently dormant) seed was recorded. Potential maximum germination was determined by adding the 21-d actual germination and the remaining firm seed. Germination data were converted to percentages before analysis. Percentage data were not transformed before analysis because data residuals were normally distributed (PROC UNIVARIATE; SAS Institute, 1999). The experimental design was a factorial arrangement of treatments (species, seed form, and water potential) in a randomized block design with four blocks. The experiment was repeated twice.

Root and shoot lengths were determined for five seedlings chosen at random from each germination box at the 7-d count. Seedling roots were preserved in a 50% formalin–propionic acid–ethanol solution for later analysis. Wet-mounts of seedling roots were made by staining with 5% rose bengal (Saha et al., 1988) and squashing onto microscope slides. A microscope fitted with an ocular micrometer was used to measure seedling root-hair length. Root-hair length was determined by measuring five randomly chosen root hairs from a seedling grown on each water potential treatment.

Data for 7-d normal germination, 21-d normal germination, potential maximum germination, seedling root and shoot lengths, and seedling root-hair length were analyzed as a facto-

rial design analysis of variance by PROC MIXED (SAS Institute, 1999). Fixed effects were species, seed form, water potential treatment, and their interactions. Blocks within run were random effects. Linear, quadratic, and cubic effects were obtained for the water potential treatment of each species by orthogonal polynomials (SAS Institute, 1999). Mean separations of seed forms was made by a t test at $P \leq 0.05$.

RESULTS

The actual percentage 7- and 21-d seed germinations varied with species \times water potential and species \times seed form interactions ($P \leq 0.05$). The species \times water potential interactions were due to yellow bluestem having strong quadratic effects ($P < 0.001$) in comparison with the other species having strong linear effects ($P < 0.001$; Fig. 1 and Fig. 2). These interaction also became insignificant ($P > 0.05$) when data for yellow bluestem were removed from the data set. The slow germination of debearded and intact spikelets of native species and the rapid germination of yellow bluestem debearded and intact spikelets likely accounted for the species \times seed form interaction at the 7-d germination period (Fig. 3). Additionally among the native grasses, sand bluestem had the highest germination of caryopses at 7 d. For the 21-d germination period, the species \times seed form interaction was due to the lack of complete germination of yellow bluestem caryopses. At 21 d, yellow bluestem caryopses germinated at 63% compared with 83% for debearded and 74% for intact spikelets (Fig. 4). Simi-

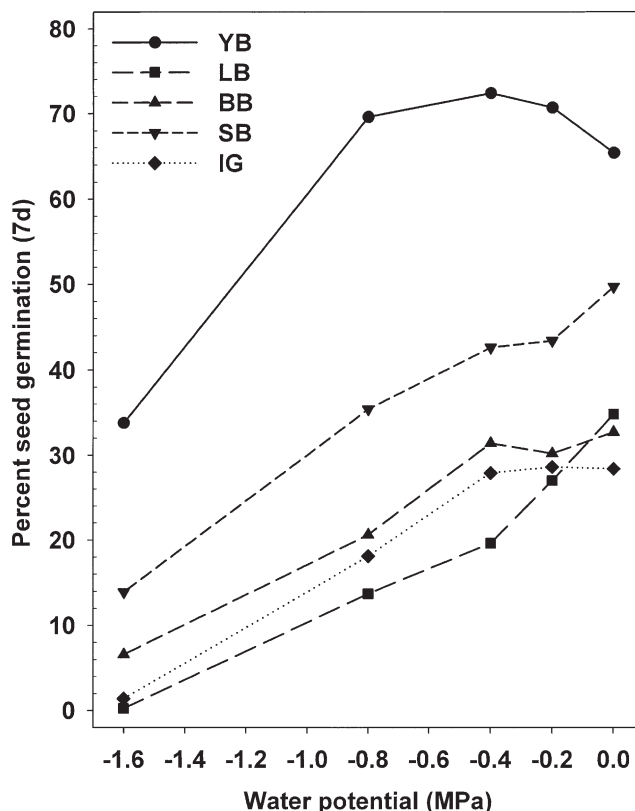


Fig. 1. Percentage 7-d seed germination of five chaffy-seeded grasses germinated at five different water potentials. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. SE of means is 4.7%.

larly, this interaction became insignificant ($P > 0.05$) when data for yellow bluestem were removed from the data set.

The potential maximum germination varied with species \times seed form interactions ($P \leq 0.05$). This interaction was due to the lack of firm seed associated with caryopses. Caryopses averaged 9% firm seed compared with 53% for debearded spikelets and 59% for intact spikelets. Potential maximum germination averaged 58% for caryopses and 96% for both debearded and intact spikelets.

Root lengths of seedlings that had germinated in 7 d varied with species \times water potential and species \times seed form interactions ($P \leq 0.05$). The 7-d germination of little bluestem and indiagrass was near zero at -1.6 MPa water potential which precluded seedling measurements at that level. Thus, data were analyzed for seedlings germinated at 0, -0.2 , -0.4 , and -0.8 MPa water potentials. The species \times water potential interaction was likely due to yellow bluestem having strong quadratic effects ($P < 0.001$) in comparison with the other species having strong linear effects ($P < 0.001$). The general trend, however, was a decrease in root length with increasing moisture stress (Fig. 5). The species \times seed form interaction for seedling root length was primarily due to shorter than expected roots from sand bluestem debearded seed (Fig. 6; $P \leq 0.05$).

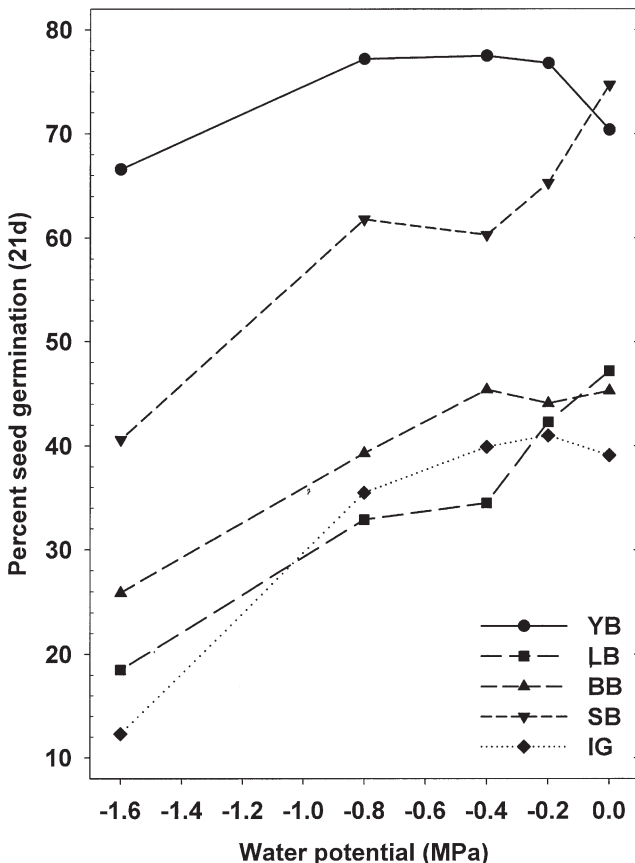


Fig. 2. Percentage 21-d seed germination of five chaffy-seeded grasses germinated at five different water potentials. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. SE of means is 3.5%.

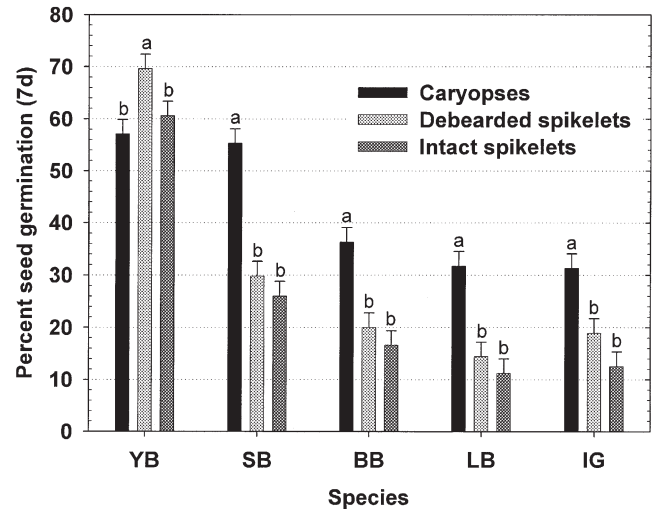


Fig. 3. Percentage seed germination of caryopses, debearded spikelets, and intact spikelets of five chaffy-seeded grasses germinated for 7 d. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. Means within species followed by the same letter are not significantly different at $P \leq 0.05$ (t test). SE of means are shown above bar.

The shoot lengths of seedlings that had germinated in 7 d varied with species \times water potential and species \times seed form interactions ($P \leq 0.05$). Similar to seedling root length, the general trend in shoot length was to decrease with increasing water potential (Fig. 7). When compared with other species, little bluestem had shorter shoots at all water potential treatments. Also similar to other data, yellow bluestem had strong linear and quadratic effect ($P < 0.001$), whereas, the other species had only strong linear effects ($P < 0.001$) which may account for the species \times water potential interaction. The species \times seed form interaction for seedling shoot length was due to the lack of differences among seed forms of little bluestem and yellow bluestem (Fig. 8; $P > 0.05$).

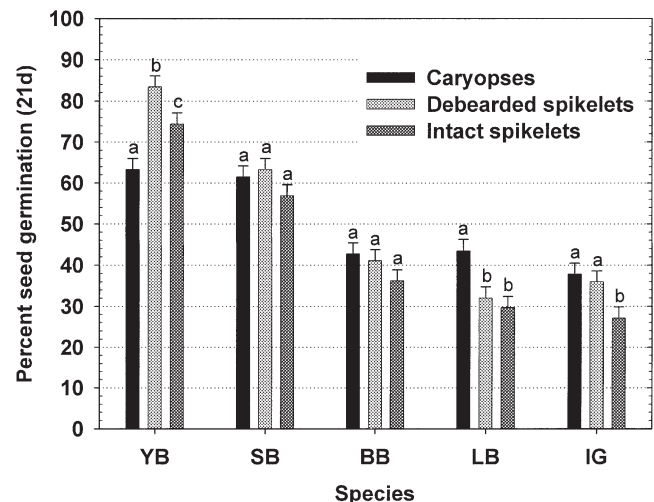


Fig. 4. Percentage seed germination of caryopses, debearded spikelets, and intact spikelets of five chaffy-seeded grasses germinated for 21 d. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. Means within species followed by the same letter are not significantly different at $P \leq 0.05$ (t test). SE of means are shown above bar.

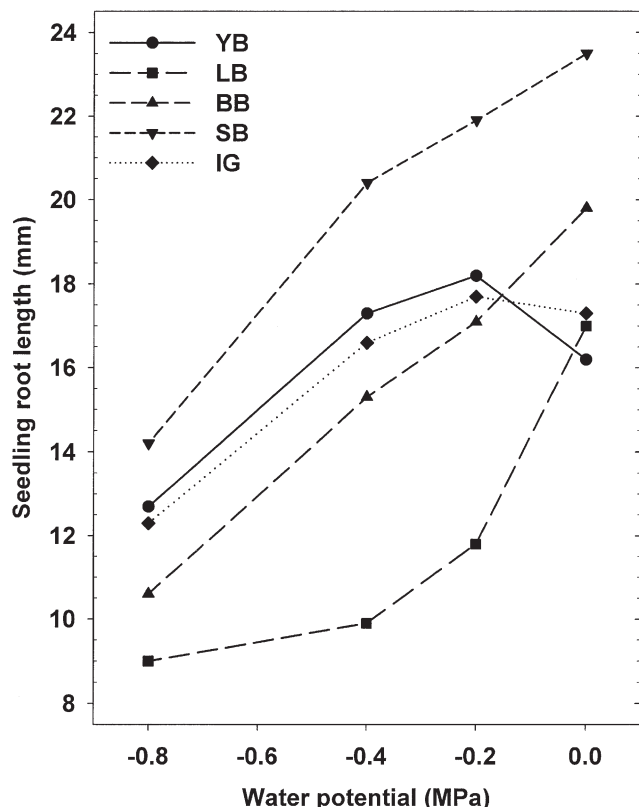


Fig. 5. Root length of seedlings of five chaffy-seeded grasses that had germinated in 7 d grown at five different water potentials. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. SE of means is 1.6 mm.

Root hair lengths of seedlings that had germinated in 7 d were not different for the main effects or their interactions ($P \geq 0.05$). The average root hair length was 96.2 μm .

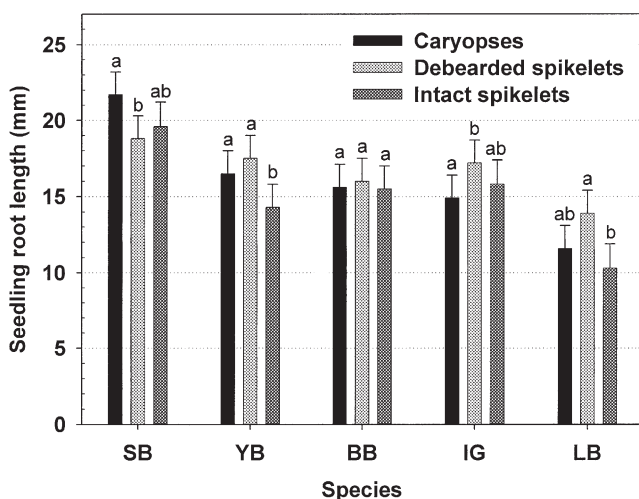


Fig. 6. Root length of seedlings that had germinated in 7 d from caryopses, debearded and intact spikelets of five chaffy-seeded grasses. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. Means within species followed by the same letter are not significantly different at $P \leq 0.05$ (t test). SE of means are shown above bar.

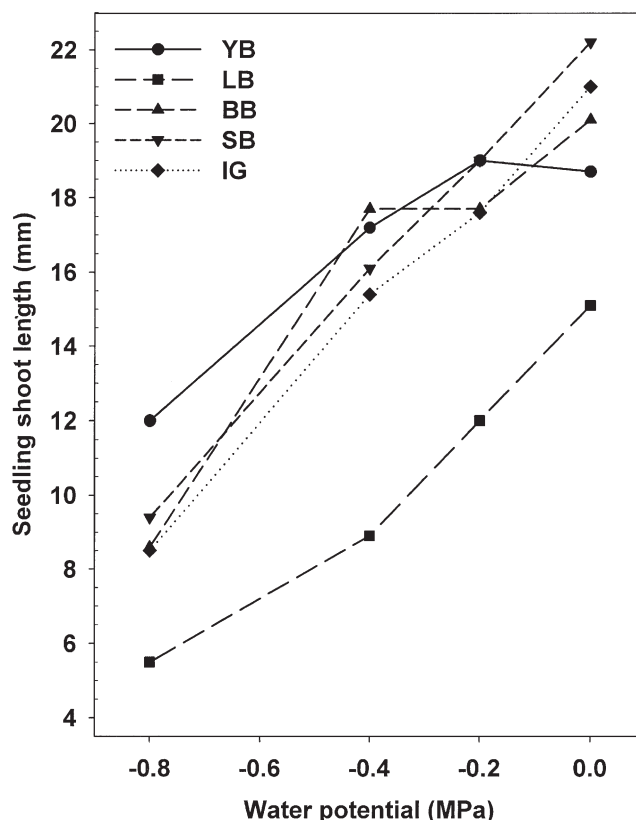


Fig. 7. Shoot length of seedlings of five chaffy-seeded grasses that had germinated in 7 d grown at five different water potentials. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. SE of means is 1.3 mm.

DISCUSSION

Within the scope of this experiment, there were no seed form \times water potential or species \times seed form \times water potential interactions ($P > 0.05$) in relation to

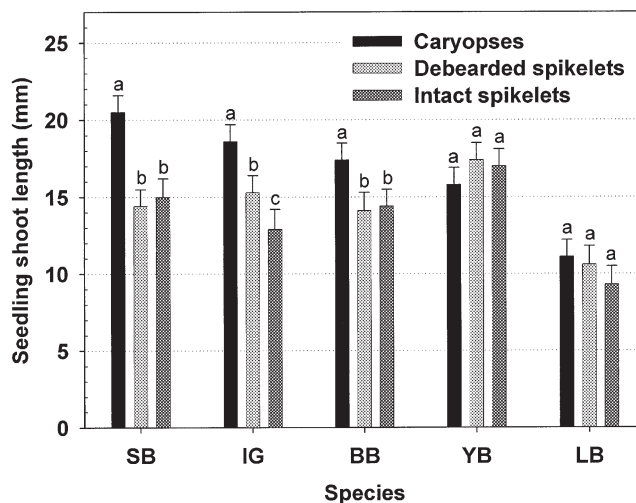


Fig. 8. Shoot length of seedlings that had germinated in 7 d from caryopses, debearded spikelets, and intact spikelets of five chaffy-seeded grasses. BB, big bluestem; IG, indiagrass; LB, little bluestem; SB, sand bluestem; YB, yellow bluestem. Means within species followed by the same letter are not significantly different at $P \leq 0.05$ (t test). SE of means are shown above bar.

7-d, 21-d, and potential maximum germination or seedling root or shoot measurements; however, there were species \times water potential and species \times seed form interactions ($P \leq 0.05$). It was not surprising to find a species \times water potential interaction, because not all species respond the same to drought stress. Sharma (1973) found differences in seed germination among five introduced and native pasture species when seeds were germinated at different water potentials. Also, Helmerick and Pfeifer (1954) found differential germination and growth response between two cultivars of winter wheat (*Triticum aestivum* L.).

The species \times water potential interaction became insignificant when data of yellow bluestem were removed from the data set. The yellow bluestem WW-Spar was originally collected in an area of Pakistan where the mean annual precipitation ranges from 300 to 400 mm. The other four species, Chet sand bluestem, Rountree big bluestem, Aldous little bluestem, and Osage indiangrass were all selected from plant materials adapted to central North America where the mean annual precipitation is much greater. Choudhuri (1968) found that seeds of *Lepidium perfoliatum* L. collected from plants adapted to low water potential environments, such as saline sites, had higher seed germination in NaCl and PEG 400 solutions at -0.25 and -0.45 MPa compared with seeds collected from nonsaline sites. In addition, Clauss and Venable (2000) concluded that populations of *Plantago insularis* Eastw. adapted to xeric environments had higher seed germination when water was available compared with populations that were adapted to mesic environments. Thus, yellow bluestem may likely be adapted to germinating in lower water potential compared with the other four species.

The lack of firm seed from caryopses accounted for the species \times seed form interaction for the potential maximum germination. Ahring et al. (1964) stated that "slight mechanical injuries not detected in laboratory tests may affect seed longevity and stand establishment under field conditions." Furthermore, Rollins and Ahring (1987) stated that caryopses "may also be more susceptible to fungal diseases in the soil." Caryopses, debearded and intact spikelets were not surface sterilized in this experiment which may have accounted for the increased mold growth and decay of nongerminating caryopses. Chemical compounds in the glumes, lemmas, and paleae may account for seed dormancy in some grasses (Ahring et al., 1975) and these compounds may also inhibit mold and other microorganisms that could possibly attack germinating caryopses.

As expected, seedling root and shoot lengths declined as water potential increased. Root and shoot lengths of seedlings that germinated at -0.8 MPa in 7 d were 62% and 45% of the control (0 MPa). Parmar and Moore (1968) reported root and shoot lengths of corn (*Zea mays* L.) seedlings that germinated at -1.01 MPa in 5 d were 55 and 32% of the control (0 MPa).

The root hair length was not affected by the osmotic agent—mannitol or the water potential of the substrate. Jackson (1965) reported mannitol-induced stimulation

to root hair length of *Agrostis alba* L. He described the cell walls of these root hairs to have a wavy appearance "due to a balance between rate of cell wall synthesis and turgor extension." This was not observed in the present experiment.

The data suggest that enough genetic variation is present to effectively select and breed cultivars with improved germination at negative water potentials. Many forage grasses are cross-pollinated and have a high degree of self sterility which results in heterozygous progeny and heterogeneous populations (Sleper, 1987). The use of recurrent restricted phenotypic selection (Burton, 1978, 1982) has been used successfully for population improvement and cultivar development in several cross-pollinated forage grasses including native grasses (Jacobson et al., 1985). The use of phenotypic selection between generations in isolated polycross nurseries allows for the removal of less desirable plants before they contribute to the next generation, which should increase the frequency of desired alleles in a resulting population (Sleper, 1987). This breeding method has been used to increase seed size and seedling vigor in sand bluestem (Kneebone and Cremer, 1955; Kneebone, 1956), forage yield of 'Pensacola' bahiagrass, *Paspalum notatum* var. *saurae* Parodi (Burton, 1982) and leaf area expansion rate in tall fescue, *Festuca arundinacea* Schreb. (Sleper, 1985). These breeding methods may also prove effective in developing populations with improved seed germination and seedling growth at low water potentials.

CONCLUSIONS

The germination of seed and early seedling growth of chaffy-seeded grasses were reduced as water potentials increased. Seed processing also affected the rate of germination and seedling growth of big bluestem, sand bluestem, little bluestem, and indiangrass in this experiment. The seed germination and seedling growth of WW-Spar yellow bluestem were least affected at low water potentials or by seed processing. No seed form \times water potential or species \times seed form \times water potential interactions were significant in relation to 7-d, 21-d, and potential maximum germination or seedling root or shoot measurements. Given the amount of genetic variation with in most cross pollinated species, this procedure would be an effective selection tool for breeding superior genotypes with improved seed germination and seedling growth at low water potentials.

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